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Letters to the Editor

Extremely Drug-Resistant *Citrobacter freundii* Isolate Producing NDM-1 and Other Carbapenemases Identified in a Patient Returning from India▽

The latest threat in multidrug resistance in Gram-negative bacteria corresponds to the emergence of NDM-1-producing isolates that have been identified in several countries worldwide. The plasmid-mediated bla_{NDM-1} gene encodes a metallo-β-lactamase (MBL) with high carbapenemase activity and was first identified in *Escherichia coli* and *Klebsiella pneumoniae* in Sweden from a patient transferred from India (14). It was later identified in different enterobacterial species from a series of patients in the United Kingdom, India, and Pakistan (8) and also from Australian and U.S. patients (3, 12).

We report here the case of an 18-year-old French female, with a history of cerebral vascular malformation that had been previously operated on, who developed an intracerebral hemorrhage during her vacation in southeast India in July 2010. She was admitted into an intensive care unit of the hospital of Pondicherry, where she received an operation. After 15 days of hospitalization in India, she was then transferred to the neurosurgical unit of a French hospital because of residual hemiplegia and aphasia. Following the occurrence of a febrile episode, a urinary infection was suspected from the urinary catheter placed in India. The catheter was removed, and the patient received an empirical treatment with amoxicillin-clavulanic acid. Urine cultures grew a *Citrobacter freundii* isolate (named STE) that was highly resistant to all β-lactams, including to carbapenems (MICs of imipenem, ertapenem, and meropenem were all >32 μg/ml). This isolate was also resistant to all aminoglycosides, sulfonamides, tetracycline, tigecycline, nitrofurantoin, and fluoroquinolones and remained susceptible to fosfomycin according to the CLSI guidelines (4), with the MIC of colistin being found at 0.5 μg/ml by the Etest method. Since the patient became apyretic, the antibiotic treatment was stopped after 3 days. No further colonization with the same bacterium was detected, neither for that patient, despite multiple screenings, nor for the other patients hospitalized simultaneously in the same unit.

Since the patient was coming from the Indian subcontinent, we focused our first PCR screening as described previously (14) and identified the bla_{NDM-1} gene in *C. freundii* STE. Then, we searched for additional β-lactamase genes, including extended-spectrum β-lactamase (ESBL) genes, MBL or AmpC genes, and class D β-lactamase genes, as described previously (5, 9, 11). Surprisingly, the search for other carbapenemase genes allowed for the identification of the bla_{VIM-4} MBL gene. In addition, the $bla_{OXA-181}$ gene encoding a variant of the class D carbapenemase OXA-48 was identified, differing by four amino acid substitutions, namely, Thr104Ala, Asn110Asp, Glu175Gln, and Ser179Ala, according to the DBL nomenclature (6) (GenBank accession number ADM26760). *In silico* analysis showed that this $bla_{OXA-181}$ gene had been identified from an *Enterobacter cloacae* isolate recovered from India (see "Nucleotide sequence accession number" below). This isolate possessed additional β-lactamase genes, including the ESBL gene $bla_{CTX-M-15}$, but also the bla_{OXA-1}, bla_{OXA-9}, bla_{OXA-10}, bla_{TEM-1}, and intrinsic bla_{CMY} genes. There are nine β-lactamase genes in that same strain, including three carbapenemase genes. In addition, the *armA* gene encoding a 16S RNA methylase was identified by using a multiplex PCR approach as described previously (1), conferring high-level resistance to all aminoglycosides.

Plasmid analysis using the Kieser method (7) allowed for the identification of five plasmids in *C. freundii* STE, with respective sizes of 40, 65, 70, 160, and 200 kb, using *E. coli* 50192 as a reference size marker (13). Using the PCR-based replicon typing (PBRT) method as described previously (2), only one plasmid type, an IncHI1 type, was identified. Mating-out assays performed as described previously (10) allowed for the identification of the bla_{NDM-1} gene on a 65-kb plasmid (pSTE-1) that was cotransferred together with two other plasmids of 70 and 160 kb. This might suggest that pSTE-1 is a mobilizable plasmid, for which attempts to determine the Inc type remained unsuccessful. The $bla_{CTX-M-15}$ gene was identified on the 70-kb plasmid that was also untypeable, together with the bla_{TEM-1} and bla_{OXA-1} genes. The *armA* gene was indentified on the 200-kb plasmid that belonged to the IncHI1 type.

This is the first example of importation of an Indian NDM-1-producing isolate in France following a hospital transfer, confirming the recent observations suggesting that the Indian subcontinent may represent an important reservoir of NDM-1 producers. This identification validates the nationwide strategy proposed recently in France to screen for multidrug-resistant bacteria, including carbapenemase producers, in patients directly transferred from a foreign hospital to a French hospital. This report indicates that unrelated carbapenemase genes may be spread along with the bla_{NDM-1} gene. It also underlines that most NDM-1 producers coproduced a 16S RNA methylase (either of the ArmA, RmtB, or RmtC type) constituting wide-spectrum aminoglycoside resistance determinants that further reduce the therapeutic options.

Nucleotide sequence accession number. The sequence of the $bla_{OXA-181}$ gene from an *E. cloacae* isolate recovered from India has been deposited in GenBank and assigned accession number HM992946.

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